Intramural Research

Scientists in the NIDDK's Intramural Research Program conduct basic, translational, and clinical biomedical research related to the Institute's mission at facilities in Bethesda, Maryland and Phoenix, Arizona. Researchers trained as post-doctoral fellows in the Intramural Research Program have become faculty members at research institutions all over the world. Intramural scientists have achieved national and international recognition for their work, several earning the Nobel Prize (see feature on Nobel laureates in this compendium). A sampling of areas under study includes: biophysics, cell biology, chemical biology and medicinal chemistry, developmental biology, molecular biology, signal transduction, structural biology, genetics and pathogenesis of diseases, and novel therapies. As in other chapters, the examples of discoveries here represent only a subset of the numerous scientific accomplishments from the past 60 years.

SNAPSHOTS OF BASIC RESEARCH DISCOVERIES

Since its inception in 1950, the NIDDK's Intramural Research Program has made a host of seminal contributions to biomedical science. Highlighted here are examples of major basic research discoveries that advanced knowledge of fundamental biologic molecules and processes, as well as the development of research methods that propelled further scientific inquiry.

Receptors: Scientists devised the first successful method to identify and measure cell surface receptors, first for adrenocorticotropic hormone and later for glucagon, insulin, growth hormone, insulin-like growth factor, thyroid-stimulating hormone, adenosine, and a wide range of gastrointestinal hormones.

DNA Gyrase and DNA Ligase: Two enzymes that are key to DNA supercoiling were discovered.

Glycoprotein Turnover: Scientists elucidated the precise conditions necessary for removal of glycoproteins from the circulation by the liver; discovered that the forms of these proteins lacking sialic acid are removed by a receptor-mediated process; and showed how the asialoglycoproteins are dismantled in liver cells. Glycoprotein breakdown products are used by cells for energy and protein synthesis.

Antibody Structure: Using X-ray crystallographic techniques to visualize the three-dimensional molecular architecture of antibodies and

SNAPSHOTS OF BASIC RESEARCH DISCOVERIES (CONTINUED)

antibody-ligand complexes, scientists made important contributions to understanding the basis of antibody recognition of foreign substances.

Gene Regulation: Scientists characterized a DNA-binding protein that regulates expression of the hemoglobin gene.

Enzyme Purification: Affinity chromatography was used to purify enzymes selectively in a single step. The method proves of great value in the isolation and purification of many biologically active proteins and polypeptides.

Pentose Phosphate Pathway: The 6-phospho-gluconate pathway of glucose breakdown, known as the pentose phosphate pathway, was elucidated. This pathway serves primarily as an important source of a coenzyme for the biosynthesis of fatty acids and steroids, and of sugars for the synthesis of nucleic acids.

Amyloid Proteins: Institute scientists were the first to elucidate the structure of an amyloid. Amyloids are abnormal forms of proteins that accumulate in disease states.

Mutagenesis: Initial research was conducted for development of the Ames test, a widely employed biological assay in which bacteria are used to determine the carcinogenicity of chemical compounds.

Biosynthesis and Physiological Effects of Polyamines: Scientists characterized a class of essential molecules known as polyamines, determining how they are synthesized, revealing how their synthesis and degradation are regulated, and uncovering their diverse physiologic functions.

Mechanism of Penicillin Action: Research led to the discovery that penicillin acts by impairing bacterial cell wall synthesis.

Molecular Crowding: The presence of high concentrations of macromolecules in cells and tissues was shown to have enormous effects on biochemical reactions.

Proteins, DNA, and Genes: Intramural researchers have determined the structure of proteins important in health and disease, deciphered the genetic code, and illuminated how segments of DNA can control activity of nearby genes. See descriptions later in this chapter of these and other key discoveries about proteins, DNA, and genes.

CLINICAL RESEARCH

Diabetes: NIDDK intramural scientists have made important contributions to diabetes research. Among their accomplishments was the delineation of the first several steps in the action of the hormone insulin, including the binding of insulin to its receptor (the protein on which it docks on the surface of cells), receptor autophosphorylation, and insulin-mediated phosphorylation of cellular proteins. They also pioneered the line of investigation leading to the principle of "down regulation" of insulin action, a concept that became widely applied to many other cell-hormone systems. Additionally, intramural researchers illuminated the post-translational modification of the insulin receptor, and alterations of the insulin receptor in human disease states—including the novel recognition of diseases associated with extreme insulin resistance. More recently, intramural researchers performed the first islet cell transplant in the United States to achieve prolonged euglycemia. Scientists in the Intramural Research Program also discovered exendin-4, a protein component of Gila lizard venom related to the hormone GLP-1 (see Diabetes, Endocrinology, and Metabolic Diseases chapter). A synthetic version of exendin-4 called exenatide was approved in 2005 as a therapy for type 2 diabetes. The medication promotes release of insulin in response to food and helps slow digestion,

making people feel full longer. NIDDK intramural research is also elucidating the function of dendritic cells and regulatory T cells (Tregs) in mediating immune "tolerance"—preventing the immune system from attacking the body's own tissues. This process goes awry in autoimmune diseases such as type 1 diabetes.

Phoenix Epidemiology and Clinical Research Branch: Established in 1963, the Branch studies the causes of type 2 diabetes as it occurs among Pima Indians of the Gila River Indian Community in Phoenix, Arizona. This population of American Indians has the highest reported prevalence of diabetes of any population in the world. Working closely with Pima Indian volunteers, the Branch has made substantial progress in identifying physiologic and genetic determinants of diabetes risk factors. The Branch also plays a pivotal role in the recruitment of Pima Indians and other American Indian populations in clinical studies that also involve extramural NIDDK-supported sites, such as the Diabetes Prevention Program clinical trial and the ongoing Diabetes Prevention Program Outcomes Study, the Look AHEAD ("Action for Health in Diabetes") clinical trial, and the Family Investigation of Nephropathy and Diabetes study. Studies often are conducted in collaboration with the Indian Health Service.

Parathyroid Hormone and Calcium Metabolism: Intramural scientists have made ground-breaking discoveries in the fields of endocrine and metabolic diseases. For example, one major set of advances by intramural researchers was the isolation of parathyroid hormone; determination of its structure; investigation of the role of this hormone, calcitonin, and vitamin D in calcium metabolism and calcium-related disorders; and characterization of molecular defects associated with pseudohypoparathyroidism. These researchers additionally helped to develop a program that achieved extraordinary success in treating patients with hyperparathyroidism. Research in this area has led to new treatments to correct abnormal calcium levels that are common in patients suffering from rare diseases of the parathyroid glands, including parathyroid cancer (see also the Diabetes, Endocrinology, and Metabolic Diseases chapter).

GTP-binding proteins: Another landmark in biomedical research was the discovery of GTP-binding ("G") proteins—molecules that, when working improperly, play key roles in numerous diseases such as diabetes, cardiovascular defects, and certain forms of cancer. Research to reveal the critical role of guanosine triphosphate (GTP) in regulating the activity of a variety of hormone receptors led to the identification of G proteins—proteins that regulate adenylate cyclase stimulation and inhibition by hormones. Such regulation is central to the transduction of hormone signals across the cell membrane. Further studies by former NIDDK Director Dr. Allen Spiegel and colleagues identified activating and inactivating mutations of G-s-alpha, a subunit of the major human G protein.

Steroid Research: Intramural scientists pioneered several fields of steroid research, including pleiotropic effects of steroid hormones. The rate-limiting step in cortisol metabolism was delineated, and the effects of thyroid hormone on this reaction were explained.

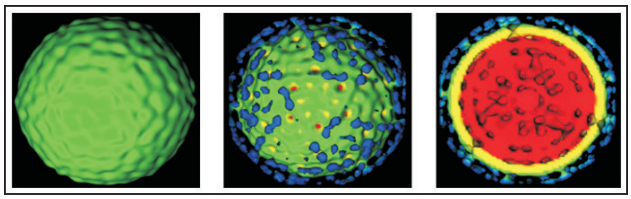
Hormone Treatment for Radiation Exposure: Intramural researchers collaborated on studies that introduced hormone treatment to thwart the development of thyroid nodules and cancer propagated by radiation fallout. The programs for the prevention and treatment of radiation-related cancers developed by these scientists have become standard care procedures for victims exposed to high levels of radiation during nuclear catastrophes such as Three Mile Island and Chernobyl.

Endocrine Tumors: A team of researchers, including current NIH Director Dr. Francis Collins and former NIDDK Director Dr. Allen Spiegel, identified the *MEN1* gene in 1997; mutations in this gene cause multiple endocrine neoplasia, a cancer syndrome marked by tumors in hormone-producing (endocrine) glands, including the parathyroid glands, pancreas, anterior pituitary, and other tissues. The scientists additionally discovered that *MEN1* gene variants are associated with tumor formation in a subset of patients with Zollinger-Ellison syndrome.

Lysosomal Storage Disorders: Critical discoveries over several decades by NIDDK intramural scientists and extramural researchers enabled the development of enzyme replacement therapy for these severe diseases. (See the Diabetes, Endocrinology and Metabolic Diseases chapter.)

Leptin as a Treatment for Lipodystrophy: The hormone leptin is secreted by fat cells and released in proportion to the amount of fat. Mice and people with very rare leptin gene mutations do lose weight when given the hormone, but leptin was not found to be effective in treating the vast majority of cases of human obesity, which are not caused by leptin deficiency. However, scientists in the NIDDK's Intramural Research Program identified another patient population—people with lipodystrophy—who could benefit from leptin treatment. Lipodystrophy is a rare and difficult-to-treat disorder, marked by a lack of normal fat in some areas of the body and excess fat in other areas. The disorder shares some metabolic problems with type 2 diabetes. People with lipodystrophy also have low levels of leptin. Clinical trials conducted since the early 2000s by scientists in the NIDDK Intramural Research Program, led by former NIDDK Director Dr. Phillip Gorden, and their collaborators found that leptin effectively treated all forms of lipodystrophy tested and corrected a broad range of metabolic defects observed in the patients. This research identified a new therapy for a rare disease and also demonstrates how the discovery of leptin has led—and continues to lead—to a cascade of exciting and unexpected findings with broad implications for improving health.

Hepatitis C: The NIDDK supports research to address the many forms of viral hepatitis, including a strong intramural research program that has been at the forefront of hepatitis C research (see also Digestive Diseases and Nutrition chapter). For example, in 1986, before the virus that causes hepatitis C was identified, NIDDK scientists tested the first effective treatment for chronic hepatitis C—long-term interferon therapy—which remains a standard treatment for the disease. Since then, the Institute has continued to perform rigorous clinical research aimed at improving the treatment of chronic hepatitis C.



Reconstructed images of a hepatitis C virus-like particle.
Image credit: Images courtesy of Dr. T. Jake Liang, Liver Diseases Branch, NIDDK Division of Intramural Research.

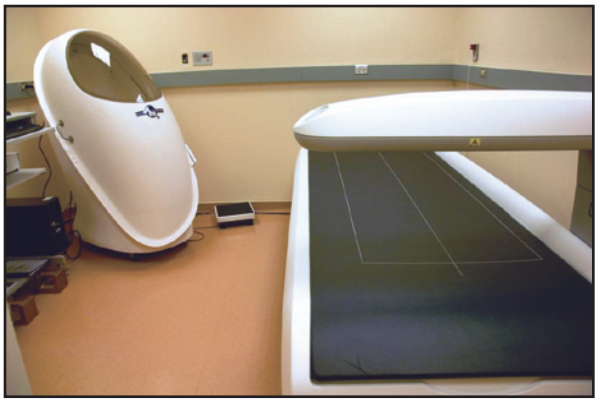
Following the discovery of the hepatitis C virus in 1989, researchers have worked to identify and characterize viral and immunological factors involved in the immune response and ability to clear the virus. A landmark achievement by NIDDK scientists was the development of one of the first cell culture systems available for studying infection by the hepatitis C virus. These cell culture systems enabled previously impossible studies of how the virus infects cells and triggers disease processes, as well as a new means of testing therapeutic antiviral agents.

An important goal for hepatitis C research from a public health standpoint is to develop a vaccine for disease prevention, a strategy that has been extremely successful for the prevention of hepatitis B. Intramural scientists have explored the potential of using hepatitis C virus-like particles as a vaccine, and are continuing this research.

Iron Metabolism: Dysregulated iron metabolism and iron overload are features of a number of human diseases. Although some genes involved in cellular iron uptake and export have been identified, very little is known about iron transport and utilization. NIDDK scientists recently reported that poly-C binding protein 1 (PCBP1) functions in both yeast and human cells as an iron chaperone and enhances the loading of iron into ferritin, a process that is essential for life in mammals.

Kidney Disease Research: Within the NIDDK Intramural Research Program, the Kidney Disease Branch is home to the Institute's research into the role of the kidneys in maintaining fluid and electrolyte balance and the causes of and treatments for kidney disease and kidney failure. Over the years, discoveries

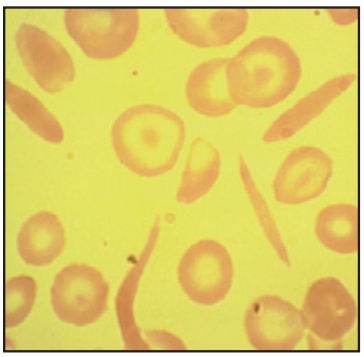
by NIDDK scientists have led to several key advances in our understanding of kidney disease, including important insights into the disease process in lupus nephritis, an inflammatory kidney disease; the identification of potential biomarkers to detect acute renal failure in its early stages and therapies to prevent and treat it; and the discovery, reported in 2008, of the relationship between genetic mutations near the *MYH9* gene locus and non-diabetic kidney disease in African Americans (see also the Kidney, Urologic, and Hematologic Diseases chapter).



The NIDDK's Metabolic Clinical Research Unit includes a body composition room where a BodPod® (left) and DXA scan machine (right) are used to measure total body composition and fat distribution. *Photo credit:* Image courtesy of Dr. Kong Chen, Director, Metabolic Research Core, NIDDK Metabolic Clinical Research Unit.

Metabolic Clinical Research Unit: In 2007, the NIDDK, in collaboration with the NIH Clinical Center, established the NIH Metabolic Clinical Research Unit (MCRU). The MCRU is designed to foster a collaborative research approach, bringing together experts from the fields of metabolism, endocrinology, nutrition, cardiovascular biology, gastroenterology, hepatology, genetics, and the behavioral sciences. The unit includes inpatient rooms, a metabolic kitchen, an exercise room, special vending machines, and a communal dining area. It also includes access to a "BodPod®" and DXA scanner. The BodPod® can measure total body density and lean and fat body mass using air displacement, while the scanner uses small doses of X-rays to calculate how much of a patient's entire body is composed of fat, muscle and bone. A signature feature of the metabolic unit is three "rapid response respiratory suites." These rooms enable researchers to measure patients' energy metabolism over 24 hours using non-invasive means. Located in the NIH Clinical Center, the unit is permitting investigators to conduct cutting-edge research on the physiology, prevention, and treatment of obesity.

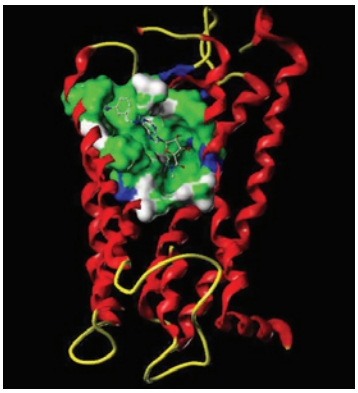
Sickle Cell Disease Research: Sickle cell disease is caused by a mutation in one of the proteins that make up hemoglobin, the oxygen-carrying component of red blood cells. This mutation results in the formation of long polymers of hemoglobin, which causes the red cells to deform into a crescent-like shape. The disease's manifestations involve nerve damage, lung and liver ailments, and periods of extreme and unrelenting bone and joint pain. Several decades of NIH studies have led to important discoveries about causes of and treatments for sickle cell disease and its complications. In studies in the 1980s, Dr. Griffin Rodgers—now NIDDK Director—and his colleagues found that the drug hydroxyurea could moderate the disease's consequences; it was approved by the FDA for this use in 1998. It remains the only drug approved for sickle cell disease. Other studies by NIDDK scientists at the NIH Clinical Center have shown that nitric oxide contributes to complications in sickle cell disease by regulating blood vessel elasticity and inhibiting platelet aggregation and adhesion. In 2009, a team of researchers, including Dr. Rodgers, reported success with a modified blood stem-cell transplant regimen to treat adult patients with sickle cell disease. Although hematopoietic stem cell transplantation represents a potential cure for sickle cell disease, finding a suitable donor remains a challenge for most patients. The NIH will continue ongoing support of research toward improved treatments and a cure for this devastating disease.



In this photograph, "sickle-shaped" cells are shown along with normal, round cells. Individuals with sickle cell disease have a genetic mutation that causes red blood cells to become sickle-shaped, which impairs the cells' ability to fit through tiny blood vessels and thus deprives tissue of oxygen. *Image credit:* Image courtesy of Dr. Griffin P. Rodgers, Director, NIDDK.

CHEMISTRY AND DRUG DEVELOPMENT

Chemistry in Nature: Research on natural products by an intramural scientist and colleagues has led to novel discoveries with major impacts on biomedical science. In one such avenue of research, the amphibian alkaloid epibatidine was found to be 200 times more potent as a painkiller than morphine. Furthermore, the 26 classes of alkaloids identified through this research have had a major impact on our knowledge of how the nervous system functions and how drugs interact with it. Exploration of another natural compound, in the early 1980s, led to the introduction of forskolin, a plant-derived chemical, as an important experimental tool used by the broader scientific community to probe the mechanism of action of drugs that act through cell-surface receptors.

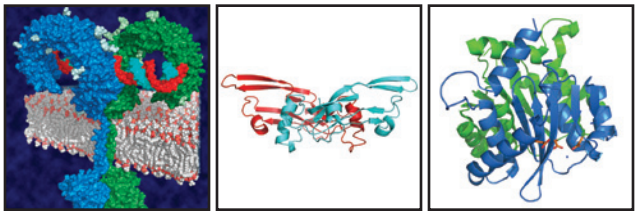


Model of a molecule activating an adenosine receptor. These models are used to study receptor signaling initiated by molecules such as caffeine.
Image credit: Image courtesy of Dr. Ken Jacobson, Chief, Molecular Recognition Section, NIDDK Division of Intramural Research.

Medicinal Chemistry: A team of intramural researchers has taken an interdisciplinary approach to studying the chemical and biological aspects of cell signaling. This research focuses on elucidating the structure and pharmacology of a specific class of cell-surface receptors and in developing drugs that act as activators or inhibitors of these molecules. With both classical chemical approaches as well as computer-aided molecular modeling and template design, these studies have begun to describe the nature of the interactions between the nucleotide and signaling molecule adenosine and its various receptors. The researchers have designed and successfully tested selective, potent activators and inhibitors for all four subtypes of adenosine receptors. These discoveries may allow future therapies to target a specific subtype of adenosine receptor, and to be more effective and have fewer side effects than those currently available, with implications for treating cardiovascular and other diseases.

PROTEINS

Structural Biology: The physical structure of proteins is an important determinant of their biological activity. One fundamental way of determining a protein's structure is a process called X-ray crystallography, in which beams of X-rays are used to develop a three-dimensional picture of the protein. Using X-ray crystallography, scientists in the NIDDK's Intramural Research Program determined the structures of several proteins involved in normal biological and disease processes. Some examples include the "toll-like" receptors, a key component of the immune system; HIV-integrase, one of the proteins of HIV; and transforming growth factor beta, a protein that plays a key part in a wide array of cellular processes. Another method of examining the shape of a protein is nuclear magnetic resonance (NMR), which uses powerful electromagnetic fields to elucidate the protein's structure. NIDDK scientists have also performed pioneering work leading to the development of new NMR-based methods of determining protein structures with great accuracy and complexity at higher resolutions. Some of these protocols have now become the widely accepted standard of carrying out protein NMR studies.

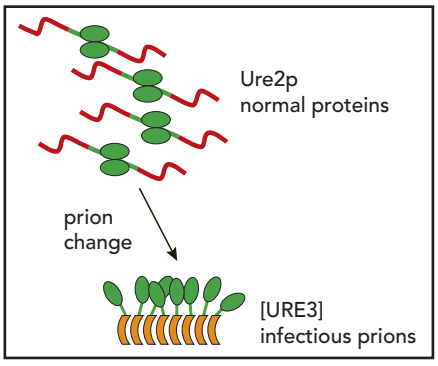


Toll-like receptor 3 | Transforming growth factor-beta | HIV integrase

Image credits: Images courtesy of Dr. David Davies, Laboratory of Molecular Biology, NIDDK Division of Intramural Research.

Protein Turnover: Much of the biochemical work within cells is accomplished by proteins, a group of molecules whose complex chemistry makes them suited to a wide variety of tasks. A protein's presence, absence, and concentration in a cell can have profound consequences. Much work centers on the rate of a protein's synthesis, but NIDDK intramural research established that the rate and timing of protein destruction (turnover) is also key to its biological properties.

Discovery of Yeast Prions: In 1994, a decades-old theory known as the "prion hypothesis" was just becoming widely accepted. In essence the theory is that fatal illnesses like scrapie in sheep, Creutzfeldt-Jakob Disease in humans, and bovine spongiform encephalopathy ("Mad Cow Disease") in cattle are all caused by an infectious "prion" form of a protein encoded by the animal's own genes. However, prions seemed limited to one protein and had been observed only in mammals. NIDDK research demonstrated that the phenomenon was much more widespread in nature, shedding light on the once-puzzling properties of the yeast genetic elements [URE3] and [PSI+]. A series of experiments tested the predictions of the prion hypothesis for [URE3], making a convincing case that it is indeed a prion form of a normal yeast protein called Ure2p. Similar experiments by others later established the same for [PSI+] as a prion form of another protein.



Several fatal diseases in humans and animals are caused by "prions"—infectious proteins that convert normal proteins into abnormal prion forms. Prions typically aggregate into structures called "amyloids." NIDDK research showed that yeast also have prions: [URE3] is a prion of the Ure2p protein. *Image credit:* Adapted with permission from Wickner RB et al. *Bioessays*. 2008; 30:955-964.

Intramural and extramural investigators have since established that three other yeast proteins—Rnq1p, PrB, and Mca1p—also form prions. This research in yeast has provided important insights into the biology and biophysics of prions.

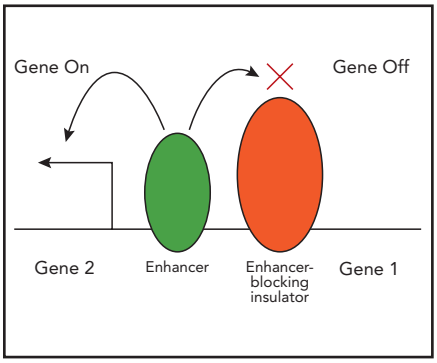
DNA AND GENES

Cracking the Genetic Code: An NIDDK intramural scientist conducted seminal experiments that led to understanding the "genetic code," the relationship between DNA, the genetic material; RNA, the messenger material; and proteins, the building blocks of cells. In 1968, he was awarded the Nobel Prize for deciphering the system used by DNA to code for the synthesis of proteins. (See also the feature on Nobel laureates in this book.)

Synthesizing DNA—and Scientists: By studying DNA replication using the T4 bacteriophage, a virus that infects bacteria, an intramural researcher identified and characterized the biochemical and molecular mechanisms essential for DNA synthesis throughout nature. In 2005, in honor of her memory and commitment to mentoring, the NIDDK established the *Nancy Nossal Fellowship Award* for intramural postdoctoral and clinical fellows.

Establishing Boundaries in the Genome: Studies of "insulator elements"—segments of DNA that mark boundaries in regions of the genome—have provided important insights into how the genome is organized and gene expression (whether a gene is turned on or off) is regulated. Insulator elements have two functions, the first of which is referred to as enhancer-blocking activity. An enhancer is a sequence of DNA that directs the cell to turn on an associated gene. If positioned near an enhancer, an insulator can prevent the enhancer's signal from being broadcast in the wrong direction and thus keep the cell from turning on genes it shouldn't. The second function of insulators is to set up a "barrier" to prevent unwanted silencing—or tight shutting off—of genes. Research in the Intramural Program has led to the identification of proteins involved in these processes and revelations into how insulators perform these two

important activities. Continued study of insulators and genome organization will lead to further insights into the regulation of genes, a process critical for health and development.



One activity of an insulator element (red) is to block another type of DNA element, an enhancer (green) from inappropriately directing a gene to turn on. *Image credit:* Adapted with permission from Gaszner M and Felsenfeld G. *Nat Rev Genet* 7: 703-13, 2006.

BIOLOGICAL MODELING

Using Math to Understand and Predict Biology: Scientists in the Laboratory of Biological Modeling use mathematics to model biological systems and learn how the systems change over time and, in doing so, pioneered the field of computational neuroscience. By investigating the behavior of oscillating signals, these scientists have applied modeling to research fields like neuronal signaling and insulin secretion from the beta cells of the pancreas.

LOOKING TO THE FUTURE

The many discoveries by NIDDK's scientists have impacted biomedical research and clinical practice. Dedicated intramural investigators will continue to pursue a broad spectrum of research to advance knowledge toward improving health.

Photo credit: Richard Nowitz, for NIDDK.